Review

Cancer Research

Efficacy and Mechanisms of Aerobic Exercise on Cancer Initiation, Progression, and Metastasis: A Critical Systematic Review of *In Vivo* Preclinical Data

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Abstract

A major objective of the emerging field of exercise–oncology research is to determine the efficacy of, and biological mechanisms by which, aerobic exercise affects cancer incidence, progression, and/or metastasis. There is a strong inverse association between self-reported exercise and the primary incidence of several forms of cancer; similarly, emerging data suggest that exercise exposure after a cancer diagnosis may improve outcomes for early-stage breast, colorectal, or prostate cancer. Arguably, critical next steps in the development of exercise as a candidate treatment in cancer control require preclinical studies to validate the biological efficacy of exercise, identify the optimal "dose", and pinpoint mechanisms of action. To evaluate the current evidence base, we conducted a critical systematic review of *in vivo* studies investigating the effects of exercise in cancer prevention and progression. Studies were evaluated on the basis of tumor outcomes (e.g., incidence, growth, latency, metastasis), dose–response, and mechanisms of action, when available. A total of 53 studies were identified and evaluated on tumor incidence ($n = 24$), tumor growth ($n = 33$), or metastasis ($n = 10$). We report that the current evidence base is plagued by considerable methodologic heterogeneity in all aspects of study design, endpoints, and efficacy. Such heterogeneity precludes meaningful comparisons and conclusions at present. To this end, we provide a framework of methodologic and data reporting standards to strengthen the field to guide the conduct of high-quality studies required to inform translational, mechanism-driven clinical trials. *Cancer Res; 76(14); 4032–50. ©2016 AACR.*

Introduction

Structured exercise training (hereto referred to as exercise) is considered an integral component of "standard of care" therapy in primary and secondary prevention of numerous common chronic conditions (1–4). In comparison, the role of exercise has received surprisingly little attention in individuals at high-risk or after a diagnosis of cancer. Over the past two decades, however, an increasing number of groups are investigating the effects of general physical activity as well as exercise in the oncology setting, a field now commonly referred to as "Exercise–Oncology" (5).

A strong body of observational evidence indicates that higher levels of self-reported exercise, physical activity, as well as cardiorespiratory fitness (i.e., objective assessment of exercise exposure) are inversely associated with the primary incidence of several forms of cancer. This evidence base is summarized by several excellent systematic reviews and meta-analyses (6–9). For example, exercise participation consistent with the national guidelines (i.e., 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity exercise per week) is associated, on average, with 25%, and 30% to 40% risk reductions in breast and colon cancers, respectively, compared with inactivity. There is also evidence of a linear dose–response relationship in prevention of breast and colon cancer. On this basis, the evidence for the exercise–prevention relationship is categorized as "convincing" for breast and colon cancer by several national agencies, and regarding as "encouraging" or "promising" for the prevention of prostate, lung, and endometrial cancers (10, 11). On the basis of this data, several phase II randomized controlled trials (RCT) were initiated, predominantly in breast cancer prevention, to investigate the effects of highly structured exercise on modulation of host-related factors (e.g., adiposity, circulating factors including sex-steroid and metabolic sex hormones, and the immune–inflammatory axis) that may underpin the exercise–cancer prevention relationship (6, 12–14). In general, exercise was associated with modest changes in markers of adiposity and select circulating factors (6, 12, 13). Whether the observed alterations are of biologic or clinical importance remains to be determined, as only one trial to

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date has investigated the exercise-induced changes in circulating factors occuring in conjunction with changes in factors/pathways in the organ/tissue of primary interest (i.e., colon crypts; ref. 15). Whether exercise reduces cancer incidence or modulates established surrogate markers of cancer incidence has not been investigated.

In patients with cancer, a steadily growing and ever diversifying series of studies indicate that, in general, exercise is a tolerable adjunct therapy associated with significant benefit across a wide range of symptom control variables both during and after primary adjuvant therapy (5, 16, 17). These data combined with the powerful inverse relationships with primary cancer incidence has led researchers to investigate whether exercise influences disease outcomes after diagnosis (18, 19). Although not nearly as mature as the evidence in the prevention setting, emerging observational data suggest that regular exercise exposure is associated with between a 10%–50% reduction in the risk of recurrence and cancer-specific death in patients with colorectal, breast, and prostate cancer (reviewed in ref. 20), compared with inactivity.

In the development of potential drug candidates, data from observational studies is insufficient to support the initiation of human trials; appropriate preclinical evidence is first required prior to human testing (21). While exercise is not a drug and exhibits a markedly different safety profile than most anticancer agents, the use of preclinical models are of critical importance to confirm the biological plausibility, establish the therapeutic window of efficacy, a biologically effective dose, and identify predictors of response (22). This evidence, combined with data from epidemiologic and molecular epidemiologic studies facilitates the design of early "signal-seeking" clinical studies and ultimately definitive RCTs. Accordingly, we conducted a critical systematic review of *in vivo* preclinical studies across the cancer continuum (i.e., prevention through metastasis). A secondary purpose was to provide recommendations to facilitate the standardization of the conduct of *in vivo* exercise–oncology studies as well as directions for future research.

Materials and Methods

Search strategy and inclusion criteria

A systematic literature search was conducted using OVID MEDLINE (1950 to May 2015), PUBMED (1962 to May 2015), and WEB OF SCIENCE (1950 to May 2015) with MeSH terms and keywords related to exercise, cancer, and animals. Text words were searched and appropriate MeSH terms were found (Supplementary Table S1). When possible, Boolean logic was used with MeSH terms to build searches. All results and reference lists were searched manually. Peer-reviewed research articles involving animals with cancer and exposed to chronic exercise (repeated bouts of more than 3 sessions) adopting either forced (i.e., treadmill running or swimming) endurance (aerobic) training or physical activity (i.e., voluntary wheel running) paradigms were considered eligible. All types of animal models of solid tumors were considered eligible including genetically predisposed models [transgenic, genetically engineered mouse models (GEMM)], orthotopic, subcutaneous, and intravenous injections, tumor transplant, and spontaneous or carcinogen-induced solid tumor models. Studies that included multiple treatments, such as study arms with dietary restrictions, were eligible, as long as it was possible to compare sedentary (control) and exercise alone groups. Studies that evaluated preneoplastic lesions (e.g., aberrant crypt foci, Apc^{Min}), or hematopoietic and ascites tumors were excluded. Only mouse and rat studies were included. Articles unavailable in English were excluded.

Study selection and classification

Four authors (K.A. Ashcraft, R.M. Peace, A.S. Betof, and L.W. Jones) assessed study eligibility by reviewing the titles and abstracts of all potential citations according to the inclusion criteria. K.A. Ashcraft, R.M. Peace, M.W. Dewhirst, and A.S. Betof extracted and interpreted data from published articles. When necessary, effort was made to contact authors and acquire publications for evaluation. Studies are summarized by type of exercise model and method of tumor initiation. Exercise characteristics are described using common prescription elements: modality, frequency, duration, intensity, and total length of intervention.

Tumor initiation versus growth

Articles were classified by the endpoints reported in the individual studies: (i) "Incidence" (tumor presence or multiplicity), (ii) "Growth" (data on tumor growth, latency or survival), and (iii) "Metastasis" (evaluation of tumors distinct from the primary tumor or developing following the common metastasis model of intravenous tumor cell injection). Study categorization was not always mutually exclusive; thus, studies that included multiple endpoints applicable to more than one categorization were included in both.

Analysis of mechanistic findings

For evaluation of mechanistic findings, an alternative classification was applied on the basis of the initiation of the exercise intervention relative to the time of tumor cell transplant or application of carcinogens. "Prevention" was defined as exercise initiated prior to tumor transplant/induction. "Progression" was defined as exercise initiated $\geq$3 days post-transplant/induction. Studies involving GEMMs were categorized as prevention studies. Distinguishing between exercise initiation and tumor cell inoculation is important because exercise-induced adaptations prior to tumor injection could "prime" the host and/or tissue microenvironment, making it physiologic or biologically distinct from tumor inoculation into a sedentary host. Mechanistic findings were classified as either intratumoral or systemic.

Interpretation and analysis

Studies were assessed for changes in tumor growth (as well as the related parameters of time to tumor-related endpoint, and tumor size/mass at the end of the study), incidence (tumor presence), and multiplicity (number of tumors/metastases). All study results that were reported to be "statistically significant" achieved $P < 0.05$ according to the authors of the original manuscripts. Preliminary analysis of included studies indicated considerable methodologic heterogeneity in all aspects of study design, end points, and efficacy. As such, it was not possible to compare the efficacy of exercise across cancer setting (i.e., incidence, progression, metastasis), cancer histology, exercise paradigm (i.e., forced vs. voluntary), or exercise dose.

Results

A total of 426 potential citations were initially identified using search terms. After secondary screening, 53 articles were deemed

eligible and underwent full review (Supplementary Fig. S1). Classification of articles was as follows: (i) incidence ($n = 24$; 45.3%; refs. 23–46), growth ($n = 33$; 62.3%; refs. 24, 26, 34, 37, 39, 40, 42, 44–69), and metastasis ($n = 10$; 18.9%; refs. 53, 55, 63, 64, 70–75).

Tumor models and exercise prescription characteristics

Included studies tested the effects of exercise on 15 different tumor types/cell lines, using six different tumor initiation methods (Supplementary Table S2). Details of the exercise prescriptions are summarized in Tables 1, 2, and 3. The most common modalities included voluntary running ($n = 22$; 41.5%; refs. 24–28, 31, 33, 35, 42, 43, 51, 52, 55, 57, 64–66, 70, 71, 73–75) forced running ($n = 25$; 47.2%; refs. 26, 29, 36–41, 44–46, 48, 49, 54, 56, 58–60, 63, 67–70, 72, 74), and swimming ($n = 10$; 18.9%; refs. 23, 30, 32, 34, 47, 50, 53, 54, 61, 62).

Effect of exercise on tumor outcomes

In incidence studies, 58% reported that exercise inhibited tumor initiation or multiplicity (23–25, 27, 28, 31, 33, 35, 36, 41, 43–46), 8% reported that exercise accelerated tumor incidence (26, 42), and 3% found null effects (29, 30, 32, 34, 37–40). In the growth category, exercise was associated with tumor inhibition in 64% of studies (24, 39, 47–54, 58–63, 65–69), while 21% (37, 40, 44, 55–57, 64) and 9% (26, 34, 42) of studies reported null and accelerated tumor growth, respectively. Finally, in the tumor metastasis category, three studies utilized models in which metastases arose from a primary tumor; of these, two reported non-significant inhibition of tumor growth (55, 64), while the other found accelerated tumor growth with exercise (53). Eight studies utilized intravenously injected tumor cell model of metastases. MacNeil and Hoffman-Goetz found that exercise did not alter retention of lung tumor cells, but increased the median number of lung metastases (74). Conversely, Jadeski and Hoffman-Goetz reported that exercise decreased retention of lung tumor cells, but had no effect on the number of lung metastases (72). One additional paper reported no difference in tumor cell retention in the lungs (71), whereas three papers reported no effect of exercise on lung metastasis (64, 70, 73). Two papers reported that exercise inhibited lung metastasis multiplicity (63) or total volume (75).

Effects on the intratumoral microenvironment

Mechanistic findings are summarized in Table 4 and Fig. 1. Studies were classified as prevention ($n = 20$; 37.7%), progression ($n = 26$; 49.1%), or metastasis ($n = 7$; 13.2%). Eight prevention studies (40%) reported significant effects of exercise in modulation of local immune response, tumor metabolism, and tumor physiology/angiogenesis. Ten progression studies (38.5%) examined mechanisms underlying the effects of exercise on tumor progression (32, 41, 42, 44, 52, 55, 57, 61, 68, 69). The mechanisms examined included multiple different factors/pathways such as apoptosis, perfusion, and immune cell infiltration. Only one metastasis study examined changes in tumor biology: Higgins and colleagues found that exercise favored a proapoptotic environment (75), reflecting changes in immune cell populations/function, tumor physiology, and signaling cascades.

Effects on systemic (host) pathways

Correlative systemic pathways examined are summarized in Table 4. Ten prevention (24, 28, 29, 40, 46–49, 59, 62), seven progression (23, 31, 37, 42, 51, 55, 64, 76), and five metastasis studies (70–72, 74, 75) reported significant effects of exercise on changes in systemic effectors, predominantly changes in factors involved in immune surveillance or metabolism.

Discussion

The findings of this critical review indicate that the current evidence base is plagued by heterogeneity in all aspects of study methodology and data reporting. Unfortunately, such heterogeneity precludes rigorous evaluation of essential questions such as the biologically effective exercise dose to modulate specific tumor pathways or inhibit tumor growth, the effects of manipulating exercise intensity or duration or the differential impact of exercise across tumor subtypes (22). Nevertheless, our review did permit identification of the most salient methodologic issues that prudent investigators may consider when designing *in vivo* preclinical exercise–oncology studies. These aspects are described in the proceeding sections and summarized in Table 5.

Heterogeneity in exercise prescription

The components of the exercise prescription being investigated in preclinical studies should mirror, as closely as possible, exercise prescription parameters tested in human studies (22). Key parameters include: (i) modality/paradigm (i.e., forced vs. voluntary), (ii) dose (i.e., intensity, session duration, frequency of training sessions/week, and length of treatment), and (iii) schedule (i.e., time of initiation).

Exercise modality (forced vs. voluntary paradigms). A foremost decision is the use of forced versus voluntary exercise paradigms. An often overlooked key point is that voluntary wheel running is a model of physical activity, whereas forced paradigms are a model of exercise training (i.e., structured and purposeful physical activity). Observational (epidemiologic) studies typically measure both physical activity and exercise, whereas efficacy-based clinical trials largely examine highly structured exercise training. The decision of which exercise modality is selected should depend on whether the investigators envisage the study findings directly informing clinical translation or plausibility/mechanisms of a clinical observation.

An important caveat to consider in all exercise paradigms is the degree of associated negative stress, as evidenced by exercise-induced increases in serum corticosterone and fecal corticosterone metabolites (77, 78), as observed in both voluntary and forced exercise paradigms. Furthermore, voluntary running may result in "addictive behavior" with negative effects on the hypothalamic–pituitary–adrenal (HPA) axis and animal health (79). Human studies have taught us that exercise induces spikes in cortisol at the beginning of exercise and immediately after bout of exercise ceases (80). Stress-induced activation of the HPA axis may accelerate tumor growth (81), and have diverse effects on the immune response (reviewed in ref. 82), thereby confounding the efficacy of exercise on tumor growth characteristics. HPA-mediated changes on the immune response could contribute to discordance between studies with respect to immune function. For example, Zhu and colleagues reported a decrease in TNFα (43) whereas Abdalla and colleagues reported an increase (23).

Table 1. Tumor incidence studies

		Methods					
				Exercise protocol			
Study	Reference	Rodent model	Tumor type/induction model	Exercise modality	Exercise prescription Freq/week | Dur | Intens | Length	Exercise initiation	Tumor incidence results
Andrianopoulos et al., 1987	25	5w old male Sprague–Dawley rats	Intestinal/DMH, i.p. q1w for 6w	Voluntary wheel running	Wheel running	1w prior to first injection	-Tumors present in 18/20 SED rats and 6/11 EX rats
Reddy et al., 1988	33	Male F344 rats	Intestinal/Subcutaneous AOM 15 mg/kg BW, q1w × 2w at 7 wk of age.	Voluntary wheel running	Wheel running for 38 weeks	4d post-AOM	-EX ↓ incidence and multiplicity of colon and small intestinal adenocarcinomas, and liver foci.
Sugie et al., 1992	35	5w old F-344 rats	Hepatocellular/15 mg/kg AOM s.c. q1w for 2w	Voluntary wheel running	38w	4d post-injection.	-Liver tumors noted in 7% of AOM treated SED only.
Ikuyama et al., 1993	28	Jc1:Wistar rats	Hepatoma/0.0177 g/day/kg BW dietary 3′-Me-DAB for 35 weeks.	Voluntary wheel running	Wheel running for 62 weeks, using food as a reward for achieving specified distances	17w prior to dietary 3′-Me-DAB	-65% reduction in tumor incidence.
Zhu et al., 2008	42	21d old female Sprague–Dawley rats	Mammary/25 or 50 mg/kg MNU, i.p.	Voluntary wheel running	Voluntary wheel running for 8w	1w post-injection	-84.5% incidence vs. 98.1%, (SED vs. EX)
Esser et al., 2009	27	C3(1)Tag mice	Prostate/transgenic mouse model	Voluntary wheel running	Wheel running for 10 weeks Data analyzed based on mice that ran >5 K or <5 K a day	10w of age	-18% (>5 K) and 55% (<5 K) of animals with high grade neoplasia at 20 weeks -57% reduction in incidence of high grade neoplasia in >5 K vs. <5 K mice
Alessio et al., 2009	24	3w old female Sprague–Dawley rats	Spontaneous tumors	Voluntary wheel running or activity box	Wheel: every other day, 24 h access throughout animals' life. Activity box (PA): 1 h in large activity box twice a week for life.	Spontaneous tumors, animals followed for life.	-During weeks 60–120, 38% of EX rats were tumor-bearing animals (vs. 42% PA rats and 54% SED rata). -At week 88, tumor multiplicity was 0.69 for EX animals, 0.75 for PA, and 0.96 for SED.
Colbert et al., 2009	26	Female heterozygous ($p53^{+/-}$): MMTV-Wnt-1 transgenic mice	Mammary/Transgenic	Voluntary wheel running and forced treadmill running	Treadmill running: TREX 1: 5x|45 min|20 m/min at 5% grade|until completion TREX 2: 5x|45 min|24 m/min at 5% grade|until completion Voluntary wheel running with 24 hour access.	11w of age	-Tumor incidence ↑ in wheel running mice by 32%
Mann et al., 2010	31	21d old female Sprague–Dawley rats	Mammary/50 mg/kg MNU i.p.	Voluntary non-motorized and motorized wheel running	Voluntary non-motorized and motorized (40 m/min) wheel running	1w post-injection	-96%, 74% and 70% incidence in controls, non-motorized and motorized mice, respectively
Zhu et al., 2012	43	21d old female Sprague–Dawley rats	Mammary/50 mg/kg MNU i.p.	Voluntary wheel running at a fixed daily distance	Three levels:WR-High: maximum 3500 m/d WR-Low: maximum 1750 m/d. SED control.	1w post-injection	-97% tumor incidence in controls, 80% tumor incidence in WR-High -Cannot compare WR-High and WR-Low, because dietary energy restriction was applied to WR-Low only
Thorling et al., 1993	36	5w old male Fischer rats	Intestinal/15 mg/kg AOM s.c. on Days 1, 4 and 8.	Forced treadmill running	5x|2 km/day|7 m/min|38w. First week was acclimatization.	3d after last injection	-EX ↓ colon neoplasia incidence, 53% vs. 78% in SED controls.
Woods et al., 1994	40	6w old male C3H/HeN mice	Mammary/2.5 × 10^5 mammary SCA-1 cells s.c. in the back.	Forced treadmill running	Treadmill running: Moderate: 7x|30 min|18 m/min at 5% grade|1w. Exhaustive: 7x|varied|18 m/min for 30 min, then 3 m/min↑ every 30 min until exhausted|1w	3d prior to injection.	-Increase in tumor incidence at Day 7 in both EX groups, but no differences in any subsequent time points.
Whittal and Parkhouse, 1996	38	21d old female Sprague–Dawley Rats	Mammary/50 mg/kg NMU i.p. at 50d of age	Forced treadmill running	Progressive training to 5x|60 min|18 m/min at 15% grade|4w	21d of age (29d prior to injection)	-Incidence/multiplicity at 24w post-NMU: 58 tumors in SED rats, 33 tumors in EX rats -No significant difference in latency or incidence

(Continued on the following page)

Table 1. Tumor incidence studies (Cont'd)

		Methods					
				Exercise protocol			
Study	Reference	Rodent model	Tumor type/induction model	Exercise modality	Exercise prescription Freq/week | Dur | Intens | Length	Exercise initiation	Tumor incidence results
Whittal-Strange et al., 1998	39	21d old female Sprague–Dawley rats	Mammary/37.5 mg/kg NMU i.p. at 50d of age, (1 day after last bout of EX)	Forced treadmill running	Progressive training to 5x|60 min|18 m/min at 15% grade|4w	21d of age (29d prior to injection)	-Incidences of carcinomas, high grade and low grade tumors were 29.2%, 10.4%, and 25% in SED, and 38%, 14.3% and 21.4% in EX
Westerlind et al., 2003	37	21d old female Sprague–Dawley rats	Mammary/25 or 50 mg/kg MNU, i.p.	Forced treadmill running	Week 1: 5x|10–15 min|30 m/min|1w. Week 2–9: 5x|30 min|23–25 m/min|8w	1w post-injection	-↑ Latency in EX (35.8d vs. 33.1d). -No difference in median tumor-free survival time was observed in the EX versus sham-EX (SHAM), nor were there any differences in multiplicity at either a high or moderate dose of MNU
Zielinski et al., 2004	44	6–8w old female BALB/c mice	Neoplastic lymphoid cells/2×10^7 EL-4 cells s.c. in the back behind the neck	Forced treadmill running	7x|3 h or until volitional fatigue|gradually increasing speed, 20–40 m/min, 5% grade|5–14d	First session immediately before injection.	-EX ↓ tumor appearance
Zhu et al., 2009	41	21d old female Sprague–Dawley rats	Mammary/50 mg/kg MNU, i.p.	Forced wheel running	Motorized running wheel; details not provided	1w post-injection	-66.7% and 92.6% incidence in EX and SED
Kato et al., 2011	29	5w old male Fischer 344 rats	Renal/5 mg/kg BW Fe-NTA i.p. once a day for 7 days, then 10 mg/kg Fe-NTA 3x/wk for 11w.	Forced treadmill running	Short-term: 15 m|8 m/min, 0%|12w. Long-term: 15 m|8 m/min, 0%|12w; then 5x|30 m|8 m/min, 0%|12w for a total of 24w training Exercise continued until 40 weeks, but at lower intensity to account for the decline in rat health.	Training done 15 m before each injection	-No differences in number of rats with nodules, nodules/rat or mean area of nodules. -Short-term EX ↑ rats with microcarcinomas, karyomegalic cells and degenerative tubules compared to SED. - Long-term EX ↓ rats with microcarcinomas, karyomegalic cells and degenerative tubules compared to short-term.
Malicka et al., 2015	45	4w old female Sprague–Dawley rats	Mammary/180 mg/kg MNU i.p.	Forced treadmill running	Low intensity (LIT): 5x|10–35 min|0.48–1.34 km/h|12w Moderate intensity (MIT): 5x|10–35 min|0.6–1.68 km/h|12w High intensity (HIT): 5x|10–35 min|0.72–2.0 km/h|12w	Immediately after MNU injection	-Incidence 64%, 67%, 40% and 43% in SED, LIT, MIT and HIT groups, respectively (*Not significant*) -Multiplicity: Rats with tumors had an average of 2.4, 1.6, 1 and 1 tumors in SED, LIT, MIT and HIT groups, respectively (*Statistics not performed.*)
Piguet et al., 2015	46	7–9w old male AlbCrePten flox/flox mice	Hepatocellular carcinoma/ Transgenic	Forced treadmill running	5w acclimation period followed by: 5x|60 m|12.5 m/min|27w	7–9w of age	-Tumor incidence 100% in SED vs. 71% in EX
Lunz et al., 2008	30	11w old male Wistar Rats	Intestinal/4 s.c. injections DMH 3 days apart.	Forced swimming with 0%, 2% or 4% BW load	Week 1–2: 5x|5–20 min|- load|2w Week 3–5: 5x|5–20 min|+ load|3w Week 6- 35: 5x|20 min|+load|30w	24 h post first injection	-No difference in tumor incidence -Aerobic swimming training with 2% body weight of load protected against the DMH-induced preneoplastic colon lesions, but not against tumor development in the rat
Paceli et al., 2012	32	Adult male Balb/c mice	Lung/1.5 mg/kg BW urethane i.p. twice, 2 days apart	Forced swimming	Aerobic: 4x|20 m|–|19w Week 1: 10 m/d to 50 m in 5 days Anaerobic: 3x|20 m (2 m swimming/2 m resting)|progressive loading of 5–20%BW|20w	Within a week after injection	-No significant effects of aerobic training on lung cancer incidence. -Aerobic training resulted in 8 lung nodules per animal vs. 52 in the control. *Not significant.* -Median control nodules was 2.0, median aerobic control nodules was 0.0. *Not significant.*
Abdalla et al., 2013	23	8w old female Balb/c mice	Mammary/1 mg/mL DMBA p.o. once weekly for 6 weeks	Forced swimming	5x|45 m|–|8 weeks Water temperature 30 ± 4°C	Same as tumor initiation	-EX ↓ tumor incidence
Sáez Mdel et al., 2007	34	50d old female Sprague–Dawley rats	Mammary/5 mg/w DMBA gastric intubation for 4w	Forced swimming	30 m/d, 5 d/w for 38–65d.	1d after appearance of first tumor	-No difference in survival time or tumor multiplicity

Abbreviations: AOM, azoxymethane; BW, body weight; d, day DMBA, 7,12-dimethylbenz(a)anthracene; DMH, 1,2-dimethylhydrazine; 3′-Me-DAB, 3′-methyl-4-dimethylaminoazobenzene; EX, exercise or activity groups; Fe-NTA, ferric nitrilotriacetate; MNU, 1-methyl-1-nitrosourea; NMU, nitrosomethylurea; q1w, once per week; SED, sedentary controls; w, weeks.

Table 2. Tumor growth studies

		Methods					
				Exercise protocol			
Study	Reference	Rodent model	Tumor type/induction model	Exercise modality	Exercise prescription Freq/week | Dur | Intens | Length	Exercise initiation	Tumor progression results
Daneryd et al., 1995	51	Female Wistar Furth rats	Leydig cell/1.5 mm^3 injection of Leydig cell sarcoma (LTW)	Voluntary wheel running	13d	Immediately after injection	-EX ↓ tumor volume by 34%.
Daneryd et al., 1995	52	Female Wistar Furth rats	Leydig cell/1.5 mm^3 injection of Leydig cell sarcoma (LTW) or Nitrosoguanine-induced adenocarcinoma s.c. into each flank	Voluntary wheel running	32d	Immediately after injection	- 13.4 g vs. 16.4 g EX vs. SED final LTW weights
Zhu et al., 2008	42	21d old female Sprague–Dawley rats	Mammary/25 or 50 mg/kg MNU, i.p.	Voluntary wheel running	Voluntary wheel running for 8w	1w post-injection	-Tumor weight 0.62 g vs. 1.16 g (SED vs. EX)
Jones et al., 2010	57	3–4w old female athymic mice	Mammary/1 $\times$ 10^6 MDA-MB-231 cells, injected orthotopically	Voluntary wheel running	Voluntary wheel running for 41–48d	2d post-implant	No change in primary tumor growth (EX ↑21%)
Yan and Demars, 2011	64	3w old male C57BL/6 mice	Lung/2.5 $\times$ 10^5/50 μl/mouse Lewis lung carcinoma cells s.c. lower dorsal region	Voluntary wheel running	Primary tumors excised at 1 cm diameter, access to wheels continued for additional 2w.	9w before tumor implantation	-No difference in tumor cross-sectional area and tumor volume.
Jones et al., 2012	55	6–8w old male C57BL/6 mice	Prostate/5 $\times$ 10^5 mouse prostate C-1 cells, orthotopically	Voluntary wheel running	Wheel running for 8 weeks	14d after tumor transplant	-Primary tumor growth was comparable between groups
Goh et al., 2014	66	18 m old Balb/cBy mice	Mammary/1 $\times$ 10^4 cells in 4th mammary fat pad	Voluntary wheel running	Voluntary wheel running for 90 days	60 days prior to tumor transplant, followed by 30 days post-transplant	-Inverse relationship between distance run and final tumor mass
Betof et al., 2015	65	Female Balb/c or female C57Bl/6 mice	Mammary/5 $\times$ 10^5 4T1-luc or 2.5 $\times$ 10^5 E0771 cells in dorsal mammary fat pad	Voluntary wheel running	Voluntary wheel running beginning either 9 weeks prior to tumor transplant, or at the time of tumor transplant	SS: SED before and after transplant RS: EX for 9 weeks prior to transplant; SED after transplant SR: SED before transplant; EX after transplant RR: EX 9 weeks prior to transplant, continuing after transplant	- Growth rates of SS and RS were similar, and growth rates of SR and RR were similar - EX slowed tumor growth compared to SED (both tumor models)
Alessio et al., 2009	24	3w old female Sprague–Dawley rats	Spontaneous tumors	Voluntary wheel running or activity box	Wheel: every other day, 24 h access throughout animals' life. Activity box (PA): 1 h in large activity box twice a week for life.	Spontaneous tumors, animals followed for life.	- EX ↓tumor growth rate
Colbert et al., 2009	26	Female heterozygous ($p53^{+/-}$): MMTV-Wnt-1 transgenic mice	Mammary/Transgenic	Voluntary wheel running and forced treadmill running	Treadmill running:TREX 1: 5x|45 min|20 m/min at 5% grade|until completion TREX 2: 5x|45 min|24 m/min at 5% grade|until completion Voluntary wheel running with 24 hour access.	11w of age	- Time to tumor size of 1.5 cm: 24.8d, 13.8d, and 19.5d in control, TREX1 and TREX2 animals. - Treadmill running led to faster tumor growth, no difference due to voluntary wheel running - Treadmill running ↓ survival
Newton et al., 1965	60	45d old Sprague–Dawley rats	Carcinoma/Equal volumes of Walker-256 cells s.c. into the right flank.	Forced treadmill running	Pre-Tumor: 50 h over 5d at 950 ft/hr. Post-Tumor: 138 h over 10d at 950 ft/hr.	5d before tumor implantation ± 4d after tumor implantation	-EX ↓ final tumor weight vs. SED control. -Early life manipulation + EX ↓ final tumor weight vs. EX alone and SED.
Uhlenbruck and Order, 1991	63	BALB/c mice	Sarcoma/2.4 $\times$ 10^4 L-1 cells s.c.	Forced treadmill running	7x|distances of 200 m/400 m/800 m|0.3 m/s|2w	4w before and 2w after injection	-200 m group ↓ tumor weight

(Continued on the following page)

Table 2. Tumor growth studies (Cont'd)

		Methods					
				Exercise protocol			
Study	Reference	Rodent model	Tumor type/induction model	Exercise modality	Exercise prescription Freq/week | Dur | Intens | Length	Exercise initiation	Tumor progression results
Woods et al., 1994	40	6w old male C3H/HeN mice	Mammary/2.5 × 10^5 mammary SCA-1 cells s.c. in the back.	Forced treadmill running	Treadmill running: Moderate: 7x|30 min|18 m/min at 5% grade|1w. Exhaustive: 7x|varied|18 m/min for 30 min, then 3 m/min↑ every 30 min until exhausted|1w	3d prior to injection.	No difference in growth rate of tumor size at time of euthanasia (two weels)
Whittal-Strange et al., 1998	39	21d old female Sprague–Dawley rats	Mammary/37.5 mg/kg NMU i.p. at 50d of age, (1 day after last bout of EX)	Forced treadmill running	Progressive training to 5x|60 min|18 m/min at 15% grade|4w	21d of age (29d prior to injection)	-Tumor growth rate at 22w post-NMU: 0.043 g/day in SED vs. 0.107 g/day in EX -Final tumor weight: (3.2 g in SED vs. 1.2 g in EX
Bacurau et al., 2000	49	12w old male Wistar rats	Carcinoma/2 × 10^7 Walker-256 cells s.c. in the flank	Forced treadmill running	5x|60 min|60% of VO2 peak|10w	8w prior to injection	-Day 15 tumor weight 1.82% vs. 19% of BW (EX vs. SED) -EX prolonged survival by 1.9 fold
Westerlind et al., 2003	37	21d old female Sprague–Dawley rats	Mammary/25 or 50 mg/kg MNU, i.p.	Forced treadmill running	Week 1: 5x|10–15 min|30 m/min|1w. Week 2–9: 5x|30 min|23–25 m/min|8w	1w post-injection	-↑ Latency in EX (35.8d vs. 33.1d). -No difference in median tumor-free survival time was observed in the EX versus sham-EX (SHAM), nor were there any differences in multiplicity at either a high or moderate dose of MNU
Zielinski et al., 2004	44	6–8w old female BALB/c mice	Neoplastic lymphoid cells/2 × 10^7 EL-4 cells s.c. in the back behind the neck	Forced treadmill running	7x|3 h or until volitional fatigue|gradually increasing speed, 20–40 m/min, 5% grade|5–14d	First session immediately before injection.	-No difference in tumor density
Jones et al., 2005	56	3–4w old female athymic mice	Mammary/Flank injection of 5 × 10^6 MDA-MB-231 cells	Forced treadmill running	5d/w|10 m/min for 10 min up to 18 m/min for 45 min|0% grade|8w	14d post-injection	-No change in tumor growth.
Bacurau et al., 2007	48	8w old male Wistar rats	Carcinoma/2 × 10^7 Walker-256 cells s.c. in the flank	Forced treadmill running	5x|30 min|85% of VO_2 max|10w	8w prior to injection	-Survival: 16d for SED, 45d for EX -EX tumors were 6.9% of final body mass vs. 17.33% for SED control.
Lira et al., 2008	58	Male Wistar rats	Carcinoma/2 × 10^7 Walker-256 cells s.c. in the flank	Forced treadmill running	5x|60 min|60–65% of VO2 peak|10w 2w pre-training period: rats ran progressively from 15 to 60 min at 10 m/min. Running was increased to 20 m/min for two weeks after injection.	8w prior to injection	-Tumor weight: 17.2 g in SED; 1.9 g in EX
Murphy et al., 2011	59	4w old C3(1)SV40Tag mice	Mammary/Transgenic (tumors began developing at 12w of age).	Forced treadmill running	6x|60 m|20 m/min at 5%|20w	4w of age	-Tumor volume: ↓ in EX at 21 and 22w.
Gueritat et al., 2014	69	10–12w old Copenhagen rats	Prostate/surgical s.c. implantation of R3327 Dunning AT1 tumor fragment	Forced treadmill running	5x|15–60 m|20–25 m/min|5w	15d after tumor implantation	- EX rats had smaller tumors at 14 and 21 days compared to SED controls - Tumor doubling time was significantly slower in EX vs. SED (6.19d vs. 8.81d)
Shalamzari et al., 2014	67	4–6w old Balb/c mice	Mammary/1 × 10^6 MC4-L2 s.c. in the flank	Forced treadmill running	-|20–40 min|6–20 m/min|15w	RTR: Sedentary before and after transplant RTE: EX after tumor transplant only ETR: EX before tumor transplant only ETE: EX before and after transplant 9w before tumor transplant, and/or 6w after transplant	- ETE had significantly slower growth compared to RTR - No difference in final tumor volume of RTE and ETR groups

(Continued on the following page)

Table 2. Tumor growth studies (Cont'd)

		Methods					
				Exercise protocol			
Study	Reference	Rodent model	Tumor type/induction model	Exercise modality	Exercise prescription Freq/week | Dur | Intens | Length	Exercise initiation	Tumor progression results
Aveseh et al., 2015	68	5w old female Balb/c mice	Mammary/1.2 × 10^6 MC4-L2 cells in right dorsal mammary fat pad	Forced treadmill running	7x|20–55 min|10–20 m/min|7w	10d after tumor transplant	-EX decreased tumor volume
Malicka et al., 2015	45	4w old female Sprague–Dawley rats	Mammary/180 mg/kg MNU i.p.	Forced treadmill running	Low intensity (LIT): 5x|10–35 min|0.48–1.34 km/h|12w Moderate intensity (MIT): 5x|10–35 min|0.6–1.68 km/h|12w High intensity (HIT): 5x|10–35 min|0.72–2.0 km/h|12w	Immediately after MNU injection	-No difference in final volume of total tumor volume
Piguet et al., 2015	46	7–9w old male AlbCrePten flox/flox mice	Hepatocellular carcinoma/ Transgenic	Forced treadmill running	5w acclimation period followed by: 5x|60 m|12.5 m/min|27w	7–9w of age	-EX decreased total volume of liver tumors
Hoffman et al., 1962	54	Wistar rats	Carcinoma/2 mL Walker 256 cell suspension s.c. into the right thigh.	Continuous running on a 20 ft runway + swimming + revolving drum	21d of EX, all EX did all 3 modalities each day:Runway: continuous running on 20 ft runway, duration and intensity not clear Swimming: increasing 20 min/day to 4 h/day Revolving drum: 5.4 mi in 12 h	Immediately after injection	-97% inhibition of tumor growth. -Tumor weight ↓ in EX group
Gershbein et al., 1974	53	Holtzman rats	Carcinosarcoma/Walker-256 tumor i.m. into both hindlimbs.	Forced swimming	10x|15 min|–|10d	Immediately after injection.	-EX ↓ tumor size. -No change in survival rates.
Baracos, 1989	50	Sprague–Dawley rats	Hepatoma/20 uL Morris hepatoma 777 s.c.	Forced swimming	Low: 5x|5 min/d, increased by 5 min/d for 3w. Medium: 5x|10 min/d, increased by 10 min/d for 3w. High: 5x|15 min/d, increased by 15 min/d for 3w.	2 Groups - 3w of swimming, tumor transplant, then 3w additional swimming. -3w of swimming beginning 3d post-transplant	-EX ↓ final tumor weight, both groups.
Radak et al., 2002	61	Adult female hybrid BDF1 mice	Solid leukemia/5 × 10^6 P-388 lymphoid leukemia cells s.c.	Forced swimming	5x|60 min|–|10w ET: training terminated at tumor implantation. EC: training continued for 18d after tumor implantation.	10w before injection.	-EC animals had slower tumor growth than ET and control, (endpoints were ∼1.24 cm^3 vs. 2 cm^3 and 2.4 cm^3)
Sáez Mdel et al., 2007	34	50d old female Sprague–Dawley rats	Mammary/5 mg/w DMBA gastric intubation for 4w	Forced swimming	30 m/d, 5 d/w for 38–65d.	1d after appearance of first tumor	-EX ↑ tumor growth by 200%.
Almeida et al., 2009	47	7w old male Swiss mice	Carcinoma/2 × 10^6 Ehrlich tumor cells s.c. in the dorsum.	Forced swimming	5x|60 min|50/80% max test|6w. Progressive load test began after 1w: load increased by 2% BW every 3 min until exhaustion.	4w before injection	-50% workload ↓ tumor weight and tumor volume (0.18 mg/g and 0.11 mm^3) vs. control (0.55 mg/g and 0.48 mm^3) -Tumor volume and weight were 270% and 280% ↑ in SED mice.
Sasvari et al., 2011	62	Adult female BDF1 mice	Sarcoma/5 × 10^6 S-180 cells s.c. injected.	Forced swimming	5x|60 m|–|10w ETT: cells injected after EX. ETC: EX (10w), cells injected, EX (18 additional days)	10w before tumor implantation.	-ETC ↓ final tumor weight vs. SED control.

Abbreviations: AOM, azoxymethane; BW, body weight; d, day; DMBA, 7,12-dimethylbenz(a)anthracene; DMH, 1,2-dimethylhydrazine; 3′-Me-DAB, 3′-methyl-4-dimethylaminoazobenzene; EX, exercise or activity groups; Fe-NTA, ferric nitrilotriacetate; MNU, 1-methyl-1-nitrosourea; NMU, nitrosomethylurea; SED, sedentary controls; w, weeks.

Table 3. Tumor metastasis studies

			Methods					
					Exercise protocol			
	Study	Reference	Rodent model	Tumor type/induction model	Exercise modality	Exercise prescription Freq/week | Dur | Intens | Length	Exercise initiation	Tumor metastasis results
Naturally occurring	Gershbein et al., 1974	53	Holtzman rats	Carcinosarcoma/Walker-256 tumor i.m. into both hindlimbs.	Forced swimming	10x|15 min|–|10d	Immediately after injection.	-Older EX rats ↑ lower abdominal and inguinal secondary tumor nodules.
	Yan and Demars, 2011	64	3w old male C57BL/6 mice	Lung/2.5×10^5/50 μl/mouse Lewis lung carcinoma cells s.c. lower dorsal region	Voluntary wheel running	Tumors excised at 1 cm diameter, access to wheels continued for additional 2w.	9w before tumor implantation	-Trend to inverse relationship between running distance and metastatic tumor yield
	Jones et al., 2012	55	6–8w old male C57BL/6 mice	Prostate/5 × 10^5 mouse prostate C-1 cells, orthotopically	Voluntary wheel running	Wheel running for 8 weeks	14d after tumor transplant	-EX ↓ tumor nodal involvement by 36%, metastases weight by 88% and number of metastases by 34% (*None were significant*)
Artificial (intravenous injection of tumor cells)	Uhlenbruck and Order, 1991	63	BALB/c mice	Sarcoma/2.4 × 10^4 L-1 cells i.v.	Forced treadmill running	7x|predetermined distances of 200–850 m|0.3–0.5 m/s|4–6w	4w before injection, followed by either rest or an additional 2w running	-EX decreased lung tumor multiplicity -Differences in running prescriptions make it difficult to determine if exercise cessation after tumor cell transplant was different from continuous exercise
	MacNeil and Hoffmann-Goetz, 1993a	74	4–5w old male C3H/He mice	Transformed fibroblasts/1.5 × 10^6 CIRAS 1 tumor cells i.v. via tail vein.	Forced treadmill and voluntary wheel running	Treadmill: 5x|30 min|15 m/min, 0%|9w. Voluntary Wheel: 24 h access to wheel for 9w. All animals remained SED after injection for 3w.	9w before injection	-No significant effect of activity on lung tumor retention. -Significant but weak correlation of distance run and multiplicity of lung metastases. -Median number of lung metastases per animal was greater in EX mice (21 vs. 10 SED control).
	MacNeil and Hoffmann-Goetz, 1993b	73	C3H/He mice	Transformed fibroblasts/3 × 10^5 CIRAS1 cells i.v.	Voluntary wheel running	3–12w.	9w prior to and/or 3w after injection	-EX had no effect on lung tumor density -EX prior to tumor injection ↑ incidence in the lowest tertile of tumor distribution vs. SED controls.
	Hoffmann-Goetz et al., 1994a	71	C3H/He-bg2J/+ and C3H/HeJ mice	Transformed fibroblasts/5 × 10^5 CIRAS 1 or CIRAS 3 radiolabeled transformed fibroblast cells i.v. via tail vein.	Voluntary wheel running	24 h access to running wheel for 9w.	9w before injection	-EX ↓ retention of radioactivity in lungs 5 min and 30 min post-injection.
	Hoffman-Goetz et al., 1994b	70	3w old female BALB/c mice	Mammary/Lateral tail vein injection of 1 × 10^4 MMT 66 cells	Forced treadmill running or voluntary wheel running	Treadmill running: 5x|30 min|18 m/min at 0%| 8w or 3w. Groups: WS: wheel mice, 24 h access for 8w, then SED for 3w. TS: treadmill mice, then SED for 3w. TT/WT: Continuous EX for 11w total. ST or SW: SED for 8w then treadmill (ST) or wheel (SW) for 3w. SS: SED for 11w total.	8w prior or 36 h after injection for 3w	-No EX effect on number of lung tumors. -TT tended to have ↑ tumor multiplicity. -ST and SW tended to have ↓ tumor multiplicity.
	Jadeski and Demars, 1996	72	4–9w old C3H/He-bg2J/+ and C3H/HeJ mice	Transformed fibroblasts/5 × 10^5 CIRAS 1 or CIRAS 3 transformed fibroblast cells i.v. via tail vein.	Forced treadmill running	5x|30 min|20 m/min, 0%|9w Acclimatization period of 1 week.	9w before injection	-EX ↓ tumor cell lung retention (44.2 vs. 52.8% control). -EX ↓ lung retention of the less aggressive CIRAS 1, no difference in CIRAS 3 cells. -EX did not alter final tumor lung metastases numbers, (subgroup evaluated 3w after EX and injection).
	Yan and Demars, 2011	64	3w old male C57BL/6 mice	Melanoma/0.75 × 10^5/200 μl/mouse B16BL/6 cells in lateral tail vein	Voluntary wheel running	Melanoma: 24 h access to wheels, continued 2w post-implantation.	9w before tumor implantation	-No difference in number of lung metastasis
	Higgins et al., 2014	75	6w old male scid-beige mice	Lung/5 × 10^5 A549-luc-C8 cells i.v. via tail vein	Voluntary wheel running	28d Mice averaged 600–1200 m/day	After lung tumors were detectable	- EX decreased tumor volume, as measured by bioluminescent imaging

Abbreviations: EX, exercise or activity groups; SED, sedentary controls.

Table 4. Mechanistic results

	Study	Reference	Tumor type	Exercise modality	Mechanistic findings		
					Intratumoral	Systemic	Potential implications
Prevention	Ikuyama et al., 1993	28	Carcinogen induced hepatoma	Voluntary wheel running	-None reported.	-EX ↓ gamma-glutamyl transpeptidase and ↑ ALP.	Reduced liver damage
	Alessio et al., 2009	24	Spontaneous tumors	Voluntary wheel running	-None reported.	-Mean serum prolactin levels were ↓ in exercising rats and ↑ in SED rats at 24 and 52w of age.	Prolactin has been associated with tumor growth
	Goh et al., 2014	66	Orthotopic mammary tumor	Voluntary wheel running	-Inverse correlation between distance run and mitotic index within tumors	-EX increased VO_2 and respiratory exchange rate	Decreased tumor cell proliferation
	Betof et al., 2015	65	Orthotopic mammary tumor	Voluntary wheel running	-EX increased colocalization of desmin and CD31 and decreased tumor hypoxia and necrosis -EX increased expression of Fas, caspase 8 and cleaved caspase 3	-None reported	Increased microvessel density and maturity, which leads to improved tumor perfusion; increased tumor cell apoptosis
	Woods et al., 1994	40	s.c. mammary tumor transplant	Forced treadmill running	-Moderate EX: ↑ phagocytic cells -Exhaustive EX: ↓ total and highly phagocytic cells	-None reported	Increased tumoricidal immune response
	Bacurau et al., 2000	48	s.c. carcinoma transplant	Forced treadmill running	-None reported	-EX ↓ glucose consumption and production, and lactate production and ↑ glutamine consumption of macrophages -EX ↓ H_2O_2 production by macrophages, and ↑ phagocytosis. -EX ↑ lymphocyte proliferation	Increased tumoricidal immune response
	Bacurau et al., 2007	49	s.c. carcinoma transplant	Forced treadmill running	-EX impairs tumor cell glucose and glutamine metabolism.	-EX, non-tumor ↑ plasma corticosterone vs. control. -EX prevents tumor-induced reduction in body weight and food intake, activation of glutamine metabolism in macrophages and lymphocytes.	Modulation of physiology
	Lira et al., 2008	58	s.c. carcinoma transplant	Forced treadmill running	-None reported.	-EX ↓ liver triacylglycerol (TAG) content compared to SED. -SED tumor animals had ↑ serum and liver TAG, and ↓ rate of VLDL secretion, apoB expression and microsomal TAG transfer protein compared to control.	Associated with reduced cachexia
	Murphy et al., 2011	59	Transgenic mammary tumors	Forced treadmill running	-None reported.	-↓ spleen weight compared to wild-type mice. -No difference in spleen weight due to EX. -EX ↓ plasma IL-6 and MP-1.	Increased tumoricidal immune response
	Kato et al., 2011	29	Carcinogen-induced renal tumors	Forced treadmill running	-None reported.	-Long-term EX and Fe-NTA ↓ renal brown droplets compared to SED, ↑ adrenal weight compared to both other groups.	Brown droplets could reflect renal damage caused by carcinogen applied. Increases in adrenal weight have been linked to psychological stress.
	Shalamzari et al., 2014	67	s.c. mammary tumor transplant	Forced treadmill running	- EX after tumor transplant decreased tumor IL-6 and VEGF - IL-6 and VEGF levels in ETR mice were not different from RTR (sedentary) mice.	-None reported	Reduced inflammation and subsequent angiogenesis
	Piguet et al., 2015	46	Transgenic hepatocellular carcinoma	Forced treadmill running	- EX decreased proliferation (Ki67 staining) in tumor nodules >15 mm^3.	- EX altered gene expression of fatty acid metabolism pathways	Altered lipogenesis. Some lipogenesis pathways are prognostic indicators following HCC surgical removal
	Malicka et al., 2015	45	Carcinogen-induced mammary tumor	Forced treadmill running	-EX increased TUNEL positive cells	- None reported	
	Almeida et al., 2009	47	s.c. carcinoma transplant	Swimming	-50% workload ↓ macrophage infiltration and neutrophil accumulation.	-80% workload induced cardiac hypertrophy vs. 50%.	Altered immune response; future analysis of macrophage subsets would be beneficial to the field
	Sasvari et al., 2011	62	s.c. sarcoma transplant	Swimming	-None reported.	-EX ↓ oxidative modification of protein in the liver as measured by protein carbonyls.	Reduced oxidative stress or improved anti-oxidant activity

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Table 4. Mechanistic results (Cont'd)

	Study	Reference	Tumor type	Exercise modality	Mechanistic findings		
					Intratumoral	Systemic	Potential implications
Progression	Daneryd et al., 1995	51	Carcinogen-induced or s.c. leydig cell transplant	Voluntary wheel running	-None reported	- EX normalized insulin sensitivity compared to SED - EX ↑ skeletal muscle metabolism - EX attenuated ↓ of reverse triiodothyronine secretion by the thyroid - EX ↑ insulin:glucagon ratio - EX ↓ corticosterone	Reflects metabolic adaptations to prevent hypo- or hyperglycemia, which may develop in cancer patients.
	Daneryd et al., 1995	52	s.c. leydig cell transplant	Voluntary wheel running	-EX 1.31-fold ↑ in tumor cell energy charge and uric acid content	-None reported.	Uric acid has since been shown to stimulate dendritic cell activation, and is elevated in tumors with anti-tumor immune responses
	Zhu et al., 2008	42	Carcinogen-induced mammary tumor	Voluntary wheel running	-EX activated AMPK -EX ↓ VEGF -EX ↓ Bcl-2, X-linked inhibitor of apoptosis pathway (XIAP) while ↑ Bax, apoptosis peptidase-activating factor-1	↓ Insulin, IGF-1, CRP, leptin and estradiol ↑ Corticosterone	EX increases cell metabolism and skews the BCL-2 family member protein profile pro-apoptotic.
	Jones et al., 2010	57	Orthotopic mammary tumor	Voluntary wheel running	-EX ↑ perfused vessels and HIF-1 protein levels	-None reported	Improved tumor perfusion, oxygenation
	Yan and Demars, 2011	64	s.c. lung tumor transplant	Voluntary wheel running	-None reported.	-Tumor presence ↑ plasma VEGF, PDGF-AB, MCP-1. -Running: ↓ plasma insulin and leptin, ↑ adiponectin, ↑ plasma VEGF.	VEGF expression has been linked to worse outcome in patients, but could instead represent improved microvessel density and vascular normalization in tumors.
	Jones et al., 2012	55	Orthotopic prostate tumor	Voluntary wheel running	-EX ↑vascularization, stabilization of HIF-1a → 40% ↑regulation of VEGF -Expression of prometastatic genes was significantly modulated in exercising animals with a shift toward reduced metastasis	-↑ activity of protein kinases within MEK/MAPK and PI3/mTOR	Improved tumor perfusion/oxygenation
	Mann et al., 2010	31	Carcinogen-induced mammary tumor	Voluntary non-motorized or motorized wheel running	-None reported	-Induced citrate synthase activity	Reflects training response
	Zhu et al., 2012	43	Carcinogen-induced mammary tumor	Voluntary wheel running at a fixed daily distance	-None reported	-↓ Insulin-like growth factor-1 (IGF-1), insulin, interleukin-6, serum amyloid protein, TNFα, and leptin -↑ IGF-binding protein 3 (IGFBP-3) and adiponectin	Increased tumoricidal immune response
	Westerlind et al., 2003	37	Carcinogen-induced mammary tumor	Forced treadmill running	-None reported	- ↑heart and soleus weights	Reflects training response
	Zielinski et al., 2004	44	s.c. neoplastic lymphoid cell transplant	Forced treadmill running	-No difference in the fluid content of tumor -EX ↓ in vessel density -EX ↓ inflammatory cells (macrophages and neutrophils) but increased lymphocytes	-None reported	Altered immune response; future analysis of macrophage subsets would be beneficial to the field
	Zhu et al., 2009	41	Carcinogen-induced mammary tumor	Forced wheel running	-Apoptosis induced via mitochondrial pathway in EX -Cell cycle progression suppressed at G(1)/S transition in EX -EX ↓ blood vessel density.	-None reported	EX promotes tumor cell apoptosis and prevents proliferations and angiogenesis

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Table 4. Mechanistic results (Cont'd)

	Study	Reference	Tumor type	Exercise modality	Mechanistic findings		
					Intratumoral	Systemic	Potential implications
	Gueritat et al., 2014	69	s.c. prostate tumor transplant	Forced treadmill running	- EX decreased tumor cell proliferation (Ki67 staining), p-ERK/ERK ratio and 8-OHdG - No difference in tumor SOD, protein carbonylation or lipid oxidattion	-None reported	ERK phosphorylation is increased by oxidative stress
	Aveseh et al., 2015	68	Orthotopic mammary tumor	Forced treadmill running	- EX shifted tumor lactate dehydrogenase expression towards the LDH1 isoenzyme - EX decreased tumor monocarboxylate transporter 1 (MCT1) and CD147 expression.	-None reported	A shift towards LDH-1 may signal reduced lactate concentrations in the tumor. Lack of MCT1 may result in the tumor cells utilizing glucose instead of lactose, and eventually starving the tumor cells. CD147 is required for MCT1 expression
	Radak et al., 2002	61	s.c. solid leukemia transplant	Swimming	-EC tumors had ↑ Ras protein compared to control. -EC tumors had ↑ I-κB protein than ET and control.	-None reported	May be associated with lymphocyte proliferation and activity, but additional analyses are needed
	Abdalla et al., 2013	23	Carcinogen-induced mammary tumor	Swimming	-None reported.	-Physical activity ↑ lymphocytes producing IFN-γ, IL-2, IL-12, and TNFα. -Physical activity promoted immune system polarization toward an antitumor Th1 response pattern profile.	Increased tumoricidal immune response
Metastasis	MacNeil et al., 1993a	74	i.v. injection of transformed fibroblasts	Forced treadmill or voluntary wheel running	-None reported.	-Wheel group ↑ splenic NK response vs. control.	Increased tumoricidal immune response
	Hoffmann-Goetz et al., 1994a	71	i.v. injection of transformed fibroblasts	Voluntary wheel running	-None reported.	-Running ↓recovery of radioactivity from liver, spleen and kidney at 30 min and 90 min post-injection.	May reflect decreased retention of circulating tumor cells in organs, thereby lowering the risk of metastasis.
	Hoffman-Goetz et al., 1994b	70	i.v. injection of mammary tumor cells	Forced treadmill running or voluntary wheel running	-None reported	-Pre-tumor EX ↑ LAK cell activity. -NK activity lower in animals that stopped EX at tumor injection.	Increased tumoricidal immune response
	Jadeski and Hoffman-Goetz, 1996	72	i.v. injection of transformed fibroblasts	Forced treadmill running	-None reported	-EX ↑ citrate synthase activity in the soleus.	Indicates a training effect developed
	Higgins et al., 2014	75	i.v. injection of lung tumor cells	Voluntary wheel running	-EX tumors had increased levels of p53, Bax and Bak. -EX tumors had increased staining for cleaved caspase-3	-EX increased serum BUN and decreased ALT, but both were still within normal ranges	Supports increased apoptosis of tumor cells

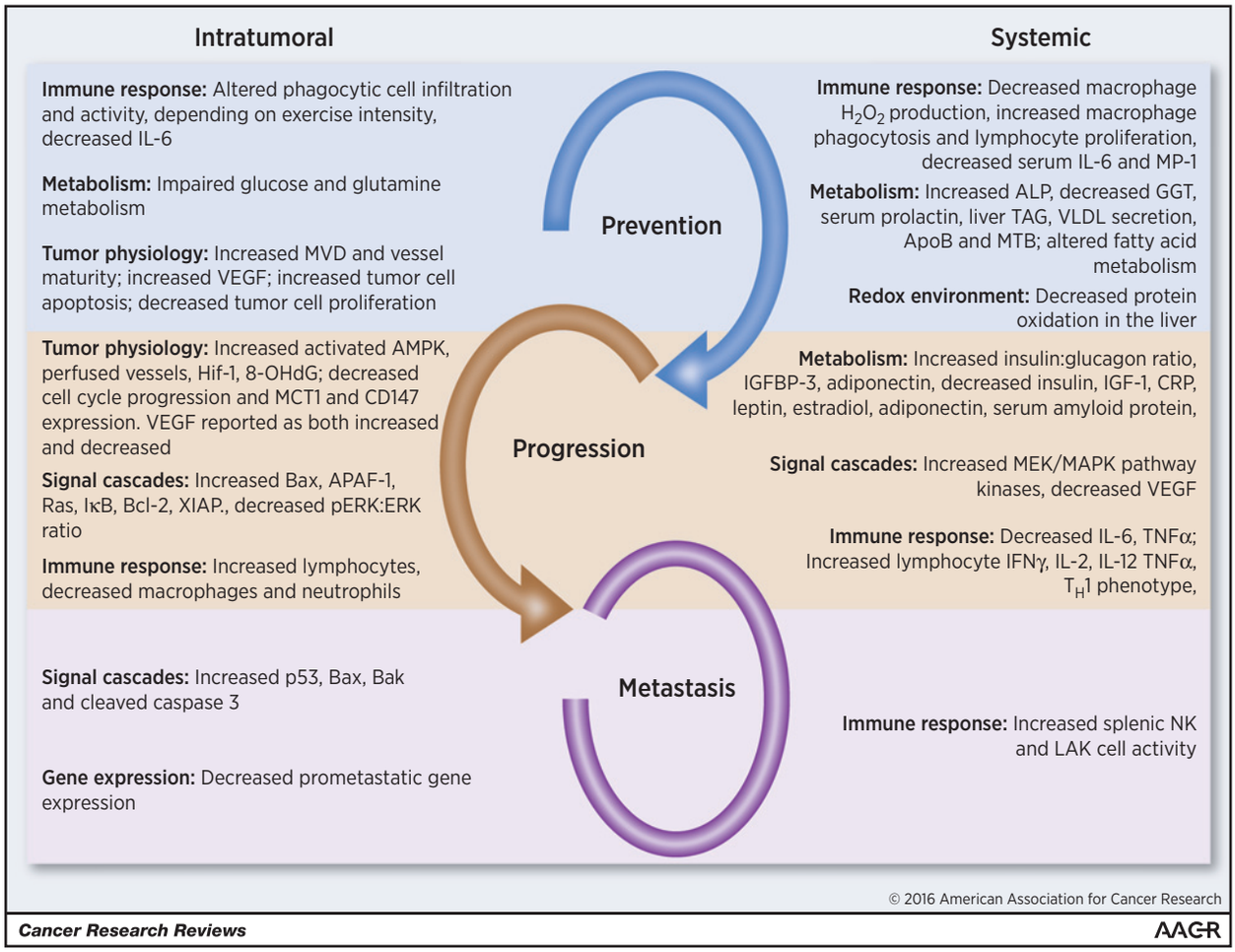

Figure 1.
Postulated intratumoral and systemic mechanisms underlying exercise oncology across the cancer continuum. Note that few reports combined measurements in tumor with systemic changes. The link between intratumoral changes and systemic changes is largely unknown.

Although changes in glucocorticoids have been seen using all exercise modes and intensities, such considerations may be especially important when investigating the effects of high-intensity or high-volume exercise treatment doses. This is relevant because, as reviewed here, studies have tested the effects of exhaustive exercise doses (ref. 40; e.g., forced swimming for 4 hours followed by 12 hours of physical activity; ref. 54), or forced swimming of animals loaded with external weights (30, 32, 47). The latter exercise paradigms may have additional safety and ethical concerns. Indeed, one study investigating the effect of forced swimming reported four animal deaths due to drowning (30). Researchers should also be cognizant of the impact of the exercise on the animals' natural circadian rhythms. Fuss and colleagues demonstrated that voluntary wheel running occurs predominantly during the animals' dark cycle (78), therefore it would be prudent to conduct exercise training during the dark cycle. Regardless of exercise prescription, corticosterone analysis would be helpful to include in all study designs; such levels could be correlated with tumor growth endpoints. In this review, three studies analyzed systemic corticosterone levels (42, 48, 51); of these, two studies reported increases in corticosterone levels, whereas the other study reported a decrease. Interestingly, tumor growth was inhibited in all studies.

Exercise dose (intensity, session duration, frequency of training, and length of treatment). Exercise at different intensities confers remarkably different physiologic and gene expression adaptations in mammals (83); however, a key question is whether these differences translate into differential effects on tumor outcomes. In clinical trials, the efficacy of different exercise intensities varying from 50% to 100% of a patient-derived physiologic parameter (e.g., age-predicted maximum heart rate, measured exercise capacity) have been investigated. Such an approach permits personalized exercise prescriptions, which is important because exercise abilities (and therefore appropriate exercise intensity) vary considerably in patients with cancer (83). Animal-specific exercise prescriptions are challenging to implement in preclinical studies, but could be important, as findings reviewed here suggest that exercise intensity may differentially modulate tumor endpoints. For example, using a transgenic model of $p53^{+/-}$ MMTV-Wnt-1,

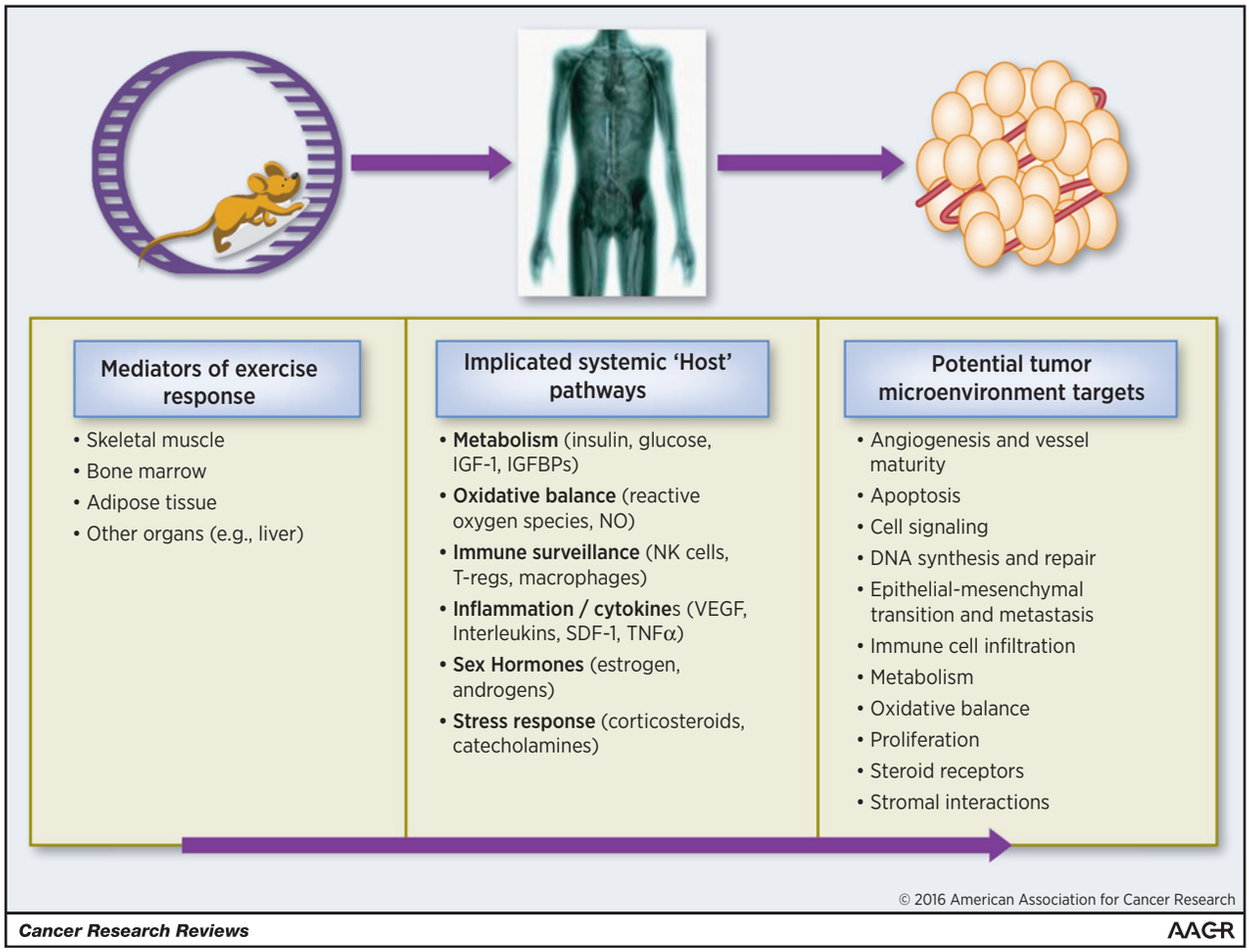

Figure 2.

Hypothesized pathways by which endurance exercise may impact tumor progression and metastasis. Key host tissues such as skeletal muscle, adipose tissue, bone marrow, and the liver mediate the effect of exercise on a variety of systemic pathways. Exercise-induced alterations in systemic and circulating factors, in turn, influences ligand availability in the tumor microenvironment, which alters cellular signaling modulating the hallmarks of cancer.

Colbert and colleagues found that exposure to forced treadmill training of different intensities accelerated tumor growth rate compared with sedentary controls, with the highest tumor multiplicity in the lowest intensity group (26). Conversely, Almeida and colleagues reported diminishing returns in the context of increasing exercise intensity using two different swimming intensities (50% or 80% of maximal workload; CT_{50} and CT_{80}, respectively, defined using a maximum load test conducted one week prior to exercise initiation; ref. 47). CT_{50} caused significant tumor growth inhibition, whereas CT_{80} was ineffective. Using a model of forced treadmill running, Woods and colleagues compared the effects of "moderate" versus "exhaustive" exercise prescriptions, with no differences in mammary tumor growth or overall incidence (40). In contrast, Malicka and colleagues reported a trend towards a negative correlation between exercise intensity [varied by progressively adjusting the speed of the treadmill (low: 0.48–1.34 km/h, moderate: 0.6–1.68 km/h, high: 0.72–2.0 km/h)] and tumor incidence and multiplicity, as well as an increase in tumor cell apoptosis across all exercise prescriptions, compared with controls (45). Kato and colleagues examined duration of prescription of forced treadmill running using a nitrilotriacetate-induced renal carcinoma model. Rats were assigned to exercise exposure for either 12 weeks or 40 weeks (29). Twelve weeks of exercise increased microcarcinoma incidence and multiplicity compared with sedentary controls with no differences (compared with control) in 40 weeks of exercise.

Schedule of exercise exposure. A critical question is whether exercise exposure prior to diagnosis improves disease outcomes compared with inactivity (prior to diagnosis). Similarly, do previously sedentary patients initiating exercise after diagnosis have superior outcomes compared with those having sedentary or decreasing exercise levels after diagnosis? Exploratory findings from epidemiologic studies suggest that timing or initiation of exercise exposure could be important (84). Two studies have empirically investigated this question: Betof and colleagues and Shalamzari and colleagues found that exercise initiated after tumor transplantation (equivalent to postdiagnosis setting) inhibited tumor growth, independent of exposure to exercise prior to

Table 5. Recommendations for preclinical exercise oncology research

Concern	Recommendation
Use of xenograft models in immunodeficit animals	Orthotopic implantation of syngeneic tumor cell lines or induction of orthotopic tumors via transgenic or chemical methods in immunocompetent animals
Poor description of exercise intervention characteristics	Describe frequency, intensity, duration, and progression, as appropriate. Avoid vague terms such as "exercise to exhaustion." Confirmation of "training" effect via muscle fiber or mitochondrial function analysis
Handling of control (sedentary) animals	Handling, social interaction, and environment should be similar to animals randomized to exercise conditions. This includes differences in cage size and social housing. If possible, animals should be acclimated to exercise, or introduced to the activity gradually.
Tail vein models of metastasis	Consider using orthotopic implantation of syngeneic tumor cell lines or transgenic models that spontaneously develop metastasis. However, tail vein models of metastasis may still be useful for assessing the effects of exercise at later time points in the metastatic cascade.
Lack of assessment of systemic and molecular mechanisms	Investigate effects on systemic mechanisms (metabolic and sex hormones, inflammation, immunity, and products of oxidation) postulated to underlie effects of exercise on tumorigenesis as well as potential mediating molecular mechanisms (e.g., cell signaling pathways, angiogenesis, metabolism, migration). Findings should be validated by the use of knock-out/knock-in transgenic animals.
Lack of assessment of tumor biology beyond tumor incidence, weight, or volume	Report on other common markers of the neoplastic phenotype (e.g., apoptosis, proliferation, microvessel density, necrosis, angiogenesis)
Lack of concern regarding the psychologic differences between voluntary physical activity vs. forced exercise	Comprehensive study on hypothalamic–pituitary–adrenal axis activation in response to different exercise prescriptions and the effects that associated hormones have on tumor progression/prevention in sedentary controls.

transplantation (65, 67). Similarly, Radak and colleagues found slower tumor growth with exercise pre- as well as post-tumor implantation in comparison with no exercise after transplant or sedentary control (61). Such findings stress the importance of maintaining exercise after diagnosis. Finally, whether the effects of exercise are different across the steps in the metastatic cascade [altered adhesion (invasion); intravasation; survival in the circulation; extravasation, and seeding at a distant site (metastatic colonization; ref. 85)] has not been investigated. Addressing this question has significant clinical implications, and further research in this area is warranted.

Common methodologic/experimental weaknesses

Several salient methodologic issues across studies were identified (see Table 5). Of these, two common issues that require particular attention regarded selection of appropriate *in vivo* tumor models, and lack of mechanistic studies.

In vivo tumor models. Preclinical oncology studies have generally focused on subcutaneous tumor implantation models. These models permit monitoring of tumor growth kinetics but do not accurately recapitulate the tissue microenvironment of the ectopic (orthotopic) or distant organ or mimic the natural evolution of cancer. In addition, blood flow to subcutaneous tumors may not reflect that of orthotopic tumors. As the benefits/risks of using subcutaneous models are beyond the scope of this review, here we will simply caution investigators against using subcutaneous tumors. This model is not a perfect surrogate for spontaneously occurring tumors.

The majority of studies reviewed herein investigated the effects of exercise on tumor growth characteristics in the primary organ site. In contrast, studies of metastasis, the primary cause of cancer-related death, has received limited attention to date. In addition, 73% of the metastasis studies we reviewed used an intravenous (tail-vein) injection of tumor cells as a model of metastasis, which evaluates the ability of tumor cells to survive in circulation and to colonize an organ but does not enable investigation of the early steps in the metastatic cascade (86). Indeed, three studies reported on tumor cell retention in the lungs following intravenous injection (71, 72, 74), with two studies reporting a decrease in tumor cell retention in exercising animals, but no change in the final number of metastases. Interestingly, MacNeil and colleagues reported that exercise did not affect tumor cell retention in the lung. These three studies differed with respect to type of exercise and number of tumor cells injected; thus no meaningful comparisons can be made between them. The impact of the methodologic differences can only be evaluated by completing the studies-side-by-side, comparing all combinations of exercise/tumor inoculation models.

An alternative, and arguably more appropriate model of metastasis, is surgical excision of the primary tumor, which stimulates spontaneous metastasis, predominantly to the lungs; only one study to date (Yan and colleagues) has adopted this model to examine exercise effects (64) and they reported a trend towards an inverse relationship between distance run and metastatic burden. Conversely, Jones and colleagues examined the metastasis response in a prostate cancer model by quantifying the mass of "non-contiguous external masses that were grossly visible independent from the primary prostate tissue." In this model, exercise was not associated with a significant reduction in the number or weight of metastases (55).

Emerging technology has provided researchers with a considerable number of *in vivo* models beyond cell line xenografts, such as patient-derived xenografts (PDX), syngeneic allografts, and GEMMs (87), as well as nonrodent models (e.g., zebrafish, *Drosophila*). The advantages and disadvantages of each model have been reviewed elsewhere (88–91). Ultimately, no one *in vivo* model will be appropriate to address all exercise–oncology questions, with model selection being contingent on the scientific question at hand as well as translational importance.

The importance of appropriate control groups. Consideration of the nature of control (nonexercising) groups should not be overlooked. To the extent possible, control animals should be exposed to the same variables as exercise counterparts including aspects related to housing, transportation to different facilities, or procedures to reinforce exercise behavior (i.e., prodding, shock). Taken even further, animals could be housed in cages with locked wheels, placed on stationary treadmills, or made to stand in very shallow pools of water, depending upon the exercise regimens

used. We stress that exposing control and experimental mice to different housing or environmental conditions is a study weakness. For example, one study housed control animals in unusually small cages (5 inches in diameter and 6 inches high) to restrict activity (54), whereas exercise treatment animals were not confined. The differences in housing size and daily movement could have either induced a stress response or altered the animals' resting metabolism, thereby affecting tumor growth. We advise researchers reviewing extant publications for planning of their own exercise oncology studies to consider whether controls were properly handled before modeling their own work off of previous studies.

Mechanistic studies/analyses. The majority of studies reviewed here examined the effects of exercise on tumor growth characteristics as evaluated by tumor volume or growth rates. While such endpoints are clearly important, subtle but important modulations of intratumoral physiologic or biological alterations can be masked. For instance, our group observed differences in perfusion and expression of key factors regulating metabolism and hypoxia, despite comparable primary tumor growth rates between exercised and sedentary animals (57). Elegant studies by McCollough and colleagues demonstrated that exercise improved blood flow and oxygen delivery to orthotopic prostate tumors in rats, but not to the normal prostate tissue in either tumor-bearing or control rats (92). These changes were reflected by decreased hypoxia within the tumor during exercise. While in-depth explications of the systemic or local molecular mechanisms underpinning the exercise–tumor prevention/progression relationship remains in its infancy, studies such as this one may set the precedent for future mechanistic studies in this field (Fig. 2). The current working hypothesis is that exercise modulates tumor progression via modulation of the host–tumor interaction (19). Tumor progression is regulated by complex, multifaceted interactions between the systemic milieu (host), tumor microenvironment, and cancer cells (93). The microenvironments of primary and metastatic tumors are subject to modulation by systemic and local growth/angiogenic factors, cytokines, hormones, and resident cells (94, 95). Factors such as hepatocyte growth factor (HGF), TNF, IL6, insulin, and leptin (96–98) have already been associated with higher risk of recurrence and cancer-specific mortality in a number of solid malignancies.(99) Clearly, manipulation of such factors by physical activity could alter aspects of the cancer continuum (100).

As reviewed here, multiple systemic factors are perturbed by exercise, including metabolism, inflammatory–immune, and reactive oxygen species–mediated pathways (101). The breadth of these factors likely contributes to the pleiotropic benefits of exercise in health and disease (102, 103) and likely the potential antitumor effects of exercise. (19) Notably, most cancer studies investigate single pathways in isolation, without consideration of overlap/synergism between pathways. Because the host–tumor interaction is modulated by numerous host-related factors and multiple pathways (100), an ideal study approach would be to investigate exercise effects on multiple pathways simultaneously (Fig. 2). This would fill in the missing gaps of the "multitargeted" effects of exercise. A recent analysis of preclinical exercise oncology studies by Pedersen and colleagues reported that only 30.7% evaluated systemic changes in animals (104). In addition, intratumoral signaling, and changes in tumor vascularity were examined by only 29.5% and 6.8%, respectively (104).

Future recommendations

With a view towards future studies, we encourage the exercise–oncology field to consider certain guidelines for preclinical exercise–oncology research (see Table 5). However, we realize that it is impractical for all exercise–oncology experiments to standardize all study procedures. Nevertheless, it may behoove the field to develop a means of quantifying the exercise prescription applied, which will then permit comparisons between studies. Such an approach is readily used in the fields of radiation oncology [i.e., the biologically equivalent dose (BED), which allows comparison of different dose/fractionation schemes (105)] and hyperthermia [i.e., the cumulative equivalent minutes at 43°C (CEM43), which standardizes the thermal killing effect of hyperthermia, regardless of variations in heating efficiencies between tumors (106)]. Establishing a biological equivalent exercise dose (BEED) would facilitate comparisons across studies as well as provide an opportunity to test elements of prescriptions such as different schedules, timing, and duration.

Exercise investigations should also strive to adopt the experimental procedures and models being utilized in the general tumor biology literature. Indeed, it appears that more recent studies reviewed here have started to utilize clinically relevant tumor models, favoring transgenic mice and orthotopic tumors over carcinogen induction. The general cancer field is also starting to favor the use of patient-derived xenograft (PDX) models. PDX have certain weaknesses, including increased heterogeneity, and a requirement for immunocompromised mice, but could represent an important step towards personalized medicine. Furthermore, PDXs do not accrue additional mutations through *in vitro* culture and tend to be slower growing than murine-derived tumor lines. Delayed growth curves may highlight slight changes in tumor growth following manipulations of exercise dose. GEMMs should also be considered, especially in mechanistic studies. A second trend in cancer biology is use of biomarkers. The discovery of blood, imaging, and/or genomic biomarker(s) to predict or monitor exercise response is of obvious importance.

Finally, researchers must be cognizant of clinical care, and design studies that reflect the clinical scenario. For example, the vast majority of the current literature investigates the effects of exercise as monotherapy. The majority of clinical scenarios would be applying exercise as an adjuvant therapy to surgery, radiation, chemotherapy, or immunotherapies. As such, it is important for the next generation of preclinical studies aiming to study tumor progression to evaluate the interaction between exercise and the pharmacodynamic or pharmacokinetic activity of conventional and novel therapies to guide the design and interpretation of clinical studies. We advise researchers to proceed with caution and carefully include all possible controls groups, because the additional therapies could introduce new confounding factors that complicate data interpretations Conversely, studies on tumor incidence may be strengthened by eliminating additional factors such as concomitant manipulation of dietary composition.

Conclusion

A sound foundation of basic and translational studies will optimize the therapeutic potential of exercise on symptom

control and clinical outcomes across the cancer continuum. Despite its importance, we found that the current evidence base is plagued by considerable methodologic heterogeneity in all aspects of study design, endpoints, and efficacy thereby precluding meaningful comparisons, and conclusions. To this end, we have provided an overview of methodologic and data reporting standards that we hope will set the platform for the next generation of preclinical studies required for the continued development and progression of exercise–oncology research.

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No potential conflicts of interest were disclosed.

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